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14. ABSTRACT Bats support a diverse ectoparasite fauna including several unique taxa that are host specific to bat species or genera. Bats are threatened worldwide by loss of habitat (Didham et al. 2007) and possibly local threats such as hunting and disease (Kamins et al. 2011). Their ectoparasites are threatened by host extinction and in some cases are believed to suffer population declines and extinction prior to the loss of the primary hosts (Bush et al. 2013). Documenting the geographic distribution of bats ticks is important because these organisms could disappear before their hosts following habitat loss or bat population crashes. In addition, there is epidemiologic and serologic evidence that bat ticks transmit pathogenic bacteria to their chiropteran hosts (Reeves et al. 2006b). Thirteen caves in the K-T Fault Ridges karst area and southern flank of the Little Quartz Ridge karst area (Miller 1996) of the Toledo District, Belize, were bioinventoried in April 2011 and May 2012 (Taylor et al. 2011, 2013). At each cave, specimens were collected by hand or with aspirators into vials of ethanol. Special effort was made to separate collections in the entrance, twilight zone, and dark zone of each cave. Where feasible, information on individual microhabitats was recorded. All caves were located near agricultural areas used by local villages, in lowland broad-leaved forest ecosystems, and due to the sensitivity of cave resources, their locations are retained by the Belize Institute of Archeology.					
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**The bat tick *Carios aztecii* (Acari: Argasidae) from Belize,
with an endosymbiotic Coxiellaceae**

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Key Words: cave, *Rickettsiella*, *Diplorickettsia*, Gammaproteobacteria, ectoparasite, Chiroptera, Toledo District.

Bats support a diverse ectoparasite fauna including several unique taxa that are host specific to bat species or genera. Bats are threatened worldwide by loss of habitat (Didham et al. 2007) and possibly local threats such as hunting and disease (Kamins et al. 2011). Their ectoparasites are threatened by host extinction and in some cases are believed to suffer population declines and extinction prior to the loss of the primary hosts (Bush et al. 2013). Documenting the geographic distribution of bats ticks is important because these organisms could disappear before their hosts following habitat loss or bat population crashes. In addition, there is epidemiologic and serologic evidence that bat ticks transmit pathogenic bacteria to their chiropteran hosts (Reeves et al. 2006b).

Thirteen caves in the K-T Fault Ridges karst area and southern flank of the Little Quartz Ridge karst area (Miller 1996) of the Toledo District, Belize, were bioinventoried in April 2011 and May 2012 (**Figure 1**) (Taylor et al. 2011, 2013). At each cave, specimens were collected by hand or with aspirators into vials of ethanol. Special effort was made to separate collections in the entrance, twilight zone, and dark zone of each cave. Where feasible, information on individual microhabitats was recorded. All caves were located near agricultural areas used by local villages, in lowland broad-leaved forest ecosystems, and due to the sensitivity of cave resources, their locations are retained by

the Belize Institute of Archeology.

Table 1. Collection records for *Carios azteci* in the Toledo District, Belize, collected in 2011 and 2012.

Date Collected	Cave/Location	Number collected
13 Apr 2011	Yok Balum	1
14 Apr 2011	Okebal Ha	3
1 May 2012	Indian Creek Cave #2	2
2 May 2012	Charles Borland Cave	1
3 May 2012	Rash Ha Cave	2

We discovered populations of argasid ticks (**Table 1**) in five of 13 caves examined: Charles Borland Cave, Indian Creek Cave # 2, Okebal Ha, Rash Ha, and Yok Balum. The Argasidae (soft tick) fauna of Belize was discussed in the New World catalog by Cooley and Kohls (1944) while the Ixodidae (hard tick) fauna was initially cataloged by Varma (1973). Neither of these publications nor the more recent extensive catalog of the cave fauna of Belize by Reddell (1981) reported *Carios azteci* (Matheson 1935) or its frequently used synonym *Ornithodoros azteci* Matheson 1935. Bioinventories in the Cayo District of central Belize by Wynne and Pleytez (2005), in Actun Chapat Cave by Reddell and Veni (1996), and in caves of the Chiquibul Cave System by Peck (1974) did not report argasid ticks.

Ticks were morphologically identified as *C. azteci* following the generic definition by Klompen and Oliver (1993) using the keys provided by Cooley and Kohls (1944), Kohls et al. (1969), and museum specimens at the US National Tick Collection, Statesboro, Georgia, USA. A voucher specimen was deposited at the US National Tick Collection (USNMENT 00862200). Five remaining ticks were bisected and DNA was extracted from half and screened for endosymbiotic and pathogenic microbes using PCR following protocols by Reeves (2005) and Foley and Reeves (2014). The intact halves were deposited in the Illinois Natural History Survey Insect Collection (Accession Numbers: 809664–809669).

Each tick was bisected and macerated with a sterile razorblade and a polypropylene pestle before digesting the remains with Proteinase K. Total DNA was extracted from individual ticks with a Maxwell 16 LEV Blood DNA Kit (Promega Corp made in Madison, Wisconsin) and resuspended in nuclease free water. Positive controls consisted of DNA from *Rickettsia prowazekii* and water was the negative control. PCR products were separated by electrophoresis on 4% agarose gels and visualized with ethidium bromide under ultraviolet light. Products were purified with a QIAquick PCR Purification Kit (Qiagen, Valencia, California). Sequencing reactions were performed with a BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, Foster City, California) using PCR primers, and excess dye was removed by ethanol precipitation. Sequences were determined using an ABI 3100 capillary sequencer (Applied Biosystems, Foster City, California), by DNA Analysis, LLC (Cincinnati, Ohio) aligned and assembled with

Chromas Lite 2.01 (Technelysium, Australia) and ClustalW (Kyoto University Bioinformatics Center, Japan), and compared to sequences in GenBank using the BLAST 2.0 program (NCBI, Bethesda, Maryland). A 1207-bp DNA sequence of the 16S rDNA gene from *Rickettsiella* or *Diplorickettsia* detected in ticks was deposited in GenBank with the accession number KT767597.

Our collections of *C. azteci* from multiple caves in Toledo District, Belize, indicate that this tick is widespread and well established in southern Belize. *Carios azteci* is probably poorly documented, because the hosts are bats and sampling in caves is not a typical habitat examined by tick biologists or veterinary parasitologists. Similar recent records for bat ticks from Nicaragua (Venzal et al. 2015) also suggest the species is more widespread. Our collections represent new national records for Belize, but *C. azteci* has probably been established in the country as long as the bat hosts were present. Although we collected numerous samples in each cave zone (entrance, twilight, and dark), *C. azteci* was only found in the dark zone (light, 0 lux), at air temperatures ranging from 23.6 to 25.7 °C, soil temperatures ranging from 21.1 to 24.9 °C, and relative humidity ranging from 88 to 92.4%.



Figure 1. Central America, showing study area in the Toledo District, southern Belize (box).

The only endosymbiont or pathogen detected in the ticks was an unnamed

Rickettsiella or *Diplorickettsia*. The 16S rDNA from these Coxiellaceae are similar and the genera are endosymbiotic or pathogenic to arthropods. They belong to the Gammaproteobacteria in the family Coxiellaceae and are related to *Coxiella burnetii* and other species reported from argasid ticks (Reeves et al. 2006a). At least one species, *Diplorickettsia massiliensis*, is also a pathogen (Subramanian et al. 2012). The diversity and biology of most Coxiellaceae remain unknown. The bacteria are consistently found in argasids, and the evolutionary relationships should be studied to understand the origin of pathogenic Coxiellaceae in relation to those that are endosymbiotic. Understanding these relationships could be a great benefit in predicting when novel pathogens will emerge as a disease agent of either bats or humans.

Ectoparasites from cave habitats are of ecological and veterinary interest as they are often poorly studied beyond the taxonomic level. Further research into the pathogens and diversity of bat parasites and diseases is an open field of research in the tropics. Some of these pathogens might even be transmitted to humans when bat ticks bite people as suggested by Loftis et al. (2005).

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Literature Cited

Bush, S.E., Reed, M., & Maher, S. 2013. Impact of forest size on parasite biodiversity: implications for conservation of hosts and parasites. *Biodiversity and Conservation* 22: 1391–1404. <http://dx.doi.org/10.1007/s10531-013-0480-x>

- Cooley, R.A., & Kohls, G.M. 1944. The Argasidae of North America, Central America, and Cuba. The American Midland Naturalist Monograph 1: 1–172.
- Didham, R.K., Tylanakis, J.M., Gemmell, N.J., Rand, T.A., & Ewers, R.M. 2007. Interactive effects of habitat modification and species invasion on native species decline. Trends in Ecology and Evolution 22: 489–496.
<http://dx.doi.org/10.1016/j.tree.2007.07.001>
- Foley, E.H., & Reeves, W.K. 2014. *Rickettsia massiliae* (Latreille) from the Azores. Journal of Agricultural and Urban Entomology 30: 25–27.
<http://dx.doi.org/10.3954/jaue14-04.1>
- Kamins, A.O., Restif, O., Ntiama-Baidu, Y., Suu-Ire, R., Hayman, D.T.S., Cunningham, A.A., Wood, J.L.N., & Rowcliffe, J.M. 2011. Uncovering the fruit bat bushmeat commodity chain and the true extent of fruit bat hunting in Ghana, West Africa. Biological Conservation 144: 3000–3008.
<http://dx.doi.org/10.1016/j.biocon.2011.09.003>
- Klompen, J.S.H., & Oliver, J.H. Jr. 1993. Systematic relationships in the soft ticks (Acari: Ixodida: Argasidae). Systematic Entomology 18: 313–331.
<http://dx.doi.org/10.1111/j.1365-3113.1993.tb00669.x>
- Kohls, G.M., Clifford, C.M., & Jones, E.K. 1969. The systematics of the subfamily Ornithodorinae (Acarina: Argasidae). IV. Eight new species of *Ornithodoros* from the Western Hemisphere. Annals of the Entomological Society of America 62: 1035–1043. <http://dx.doi.org/10.1093/aesa/62.5.1035>
- Loftis, A.D., Gill, J.S., Schriefer, M.E., Levin, M.L., Ereemeeva, M.E., Gilchrist, M.J., & Dasch, G.A. 2005. Detection of *Rickettsia*, *Borrelia*, and *Bartonella* in *Carios kelleyi* (Acari: Argasidae). Journal of Medical Entomology 42: 473–480.
<http://dx.doi.org/10.1093/jmedent/42.3.473>
- Miller, T.E. 1996. Geologic and hydrologic controls on karst and cave development in Belize. Journal of Cave and Karst Studies 58: 100–120.
- Peck, S.B. 1974. Recent studies on the invertebrate fauna and ecology of sub-tropical and tropical American caves. Association for Mexican Cave Studies Newsletter 5: 30–38.
- Reddell, J.R. 1981. A Review of the Cavernicole Fauna of Mexico, Guatemala, and Belize. Bulletin 27 of the Texas Memorial Museum. University of Texas at Austin, Austin, Texas, USA. 327 pp.
- Reddell, J.R., & Veni, G. 1996. Biology of the Chiquibul Cave System, Belize and Guatemala. Journal of Cave and Karst Studies 58: 131–138.

- Reeves, W.K. 2005. Molecular genetic evidence for a novel bacterial endosymbiont of *Icosta americana* (Diptera: Hippoboscidae). *Entomological News* 116: 263–265.
- Reeves, W.K., Loftis, A.D., Sanders, F., Spinks, M.D., Wills, W., Denison, A.M., & Dasch, G.A. 2006a. *Borrelia*, *Coxiella*, and *Rickettsia* in *Carios capensis* (Acari: Argasidae) from a brown pelican (*Pelecanus occidentalis*) rookery in South Carolina, USA. *Experimental and Applied Acarology* 39: 321–329.
<http://dx.doi.org/10.1007/s10493-006-9012-7>
- Reeves, W.K., Streicker, D.G., Loftis, A.D., & Dasch, G.A. 2006b. Serologic survey of *Eptesicus fuscus* from Georgia, U.S.A. for *Rickettsia* and *Borrelia* and laboratory transmission of a *Rickettsia* by bat ticks. *Journal of Vector Ecology* 31: 386–389.
[http://dx.doi.org/10.3376/1081-1710\(2006\)31\[386:SSOEFF\]2.0.CO;2](http://dx.doi.org/10.3376/1081-1710(2006)31[386:SSOEFF]2.0.CO;2)
- Subramanian, G., Mediannikov, O., Angelakis, E., Socolovschi, C., Kaplanski, G., Martzolf, L., & Raoult, D. 2012. *Diplorickettsia massiliensis* as a human pathogen. *European Journal of Clinical Microbiology & Infectious Diseases* 31: 365–369.
<http://dx.doi.org/10.1007/s10096-011-1318-7>
- Taylor, S.J., Heads, S.W., Jacoby, J., Hoese, G.B., Krejca, J.K., Beveridge, A.E., & Hager, K.D. 2013. 2012 Belize biospeleology expedition. INHS Technical Report 2013(43): 1–22 + appendices. <http://hdl.handle.net/2142/47550>
- Taylor, S.J., Slay, M.E., Jacoby, J., Hoese, G.B., Krejca, J.K., Slay, C.M., & Bond, J.E. 2011. 2011 Belize biospeleology expedition report. INHS Technical Report 2011(43). Subterranean Ecology Institute, Inc. and Illinois Natural History Survey, Champaign, Illinois, USA. 51 pp. <http://hdl.handle.net/2142/28585>
- Varma, M.G. 1973. Ticks (Ixodidae) of British Honduras. *Transactions of the Royal Society of Tropical Medicine and Hygiene* 67: 92–102.
[http://dx.doi.org/10.1016/0035-9203\(73\)90325-8](http://dx.doi.org/10.1016/0035-9203(73)90325-8)
- Venzal, J.M., Hernández, L.V., Labruna, M.B., & Nava, S. 2015. *Nothoaspis reddelli* Keirans & Clifford, 1975 (Acari, Ixodida, Argasidae): distribution extension. *CheckList* 11: 1–2. <http://dx.doi.org/10.15560/11.4.1698>
- Wynne, J.J., & Pleytez, W. 2005. Sensitive ecological areas and species inventory of Actun Chapat Cave, Vaca Plateau, Belize. *Journal of Cave and Karst Studies* 67: 148–157.